

PAPER • OPEN ACCESS

The quality of intact plasma membrane of bull frozen sperm in different breeds

To cite this article: A M Diansyah *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **788** 012134

View the [article online](#) for updates and enhancements.

The quality of intact plasma membrane of bull frozen sperm in different breeds

A M Diansyah¹, M Yusuf², A L Tolleng², S Surahman², M Raafi² and Sahiruddin²

¹Post-Graduate School, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea Makassar, South Sulawesi, Indonesia

²Faculty of Animal Husbandry, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea Makassar, South Sulawesi, Indonesia

Email: athhar.md13@gmail.com

Abstract. Hypoosmotic Swelling (HOS) Test is a technique to evaluate the intact plasma membrane of spermatozoa. The purpose of this study was to determine the level of intact plasma membrane in various breeds of cattle regardless of age, feed, environment and maintenance management. Semen examination was carried out microscopically. Valuable semen assessment parameter was the percentage of intact plasma membrane in the cows of Bali, Limosin, FH, Brahman, Angus and Simental. Cattles with the highest intact plasma membrane values were Simental and the lowest was FH. The results of statistical analysis on the intact plasma membrane spermatozoa showed a significant difference ($P < 0.05$) between cattle breeds. The intact plasma membrane in Simental was significantly different ($P < 0.05$) compared to Bali, Limousine and FH. However, it is not significantly different from the Brahman and Brangus cattle. Different breeds of cattle can affect the level of intact plasma membrane.

1. Introduction

Efforts to improve the appearance of cattle with high quality production capacity can be pursued through the provision and dissemination of superior livestock breeds, especially the use of superior bulls, so that the contribution of cattle to the provision of livestock origin food will be more pronounced. Advances in livestock biotechnology today are directed in the field of reproduction, one of which is Artificial Insemination (AI). Artificial insemination is a technique of inserting thawed and processed male spermatozoa into the female genital tract using a special method and tool called an insemination gun [1]. For the purpose of IB, spermatozoa can be stored in the form of fresh semen or frozen semen. Semen is diluted using diluents that contain buffers, energy sources, antibiotics and lipoproteins that can maintain their quality during storage. In the form of fresh semen, ejaculate semen can be stored for four days at 4°C with a motility of more than 40% so it is still suitable for use for IB [2].

One of the goals of reproductive technology is to determine the ability of fertility [3]. To carry out fertilization, spermatozoa must move to the place of fertilization using the energy obtained from the diluent, so that motility is often used as an indicator of spermatozoa fertility. However, the movement of spermatozoa is influenced by the integrity of the morphological structure of the spermatozoa [2].



Ranch usually assess the quality (motility, spermatozoa concentration, morphology, intact plasma membrane and viability) of semen prior to use for artificial insemination [4], and other tests have been developed [5].

Hypo-osmotic Swelling Test (HOST) is a technique to evaluate the intact plasma membrane in domestic animals including cattle [6], horses [7] and pigs [8], but it is performed on fresh semen. Testing for beef frozen semen is rarely performed. The intact plasma membrane of spermatozoa is absolutely necessary to fulfil its function as a protective organelle in the cell and a filter for the exchange of intracellular and extracellular substances. The purpose of this study was to determine the level of intact plasma membrane in various breeds of cattle regardless of age, feed, environment and maintenance management.

2. Material and methods

2.1. Material

The study was conducted in May 2020 at Laboratorium Processing Semen Fakultas Peternakan Universitas Hasanuddin, Makassar. The material used were straw, tweezers, scissor straw, waterbath [*minitube*[®]], micro pipette 100 µl and 1000µl, oven, microscope [*ZEISS*], micro-tube 1.5 ml [*Onemed*[®]], tip, object glass, cover glass [*Onelab*[®]], container straw, warmplate [*minitube*[®]], cutter straw [*minitube*[®]], frozen semen of different breeds that is Bali, Brahman, Simmental, Limmousin, Fries Holland (FH), dan Brangus from Dinas Peternakan dan Kesehatan Hewan Provinsi Sulawesi Selatan. Other materials are warm water 40°C, tissue, label, liquid nitrogen and HOST solution.

2.2. Methods

2.2.1. *Thawing*. Thawing of frozen semen by dipping straw in waterbath with temperature of 37°C for 30 seconds. Straw was dried with a tissue paper, then both ends of the straw are cut and placed in a microtube. Further, the tube was incubated on oven at 37°C for 30 minutes, then microscopical evaluation is carried out.

2.2.2. *Evaluation intact plasma membrane*. Evaluation of intact plasma membrane was carried out microscopically. Intact plasma membrane was observed by adding HOST solution (0.179g NaCl in 100 ml of aquabides) into 10 µl semen, then incubated for 30 minutes at 37°C in oven. Spermatozoa with intact plasma membranes were characterized by a circular tail and damaged sperm were characterized by a straight tail. Evaluation was carried out with a 400 times magnification microscope by counting 200 spermatozoa cells using the formula:

$$I = [C/(C+S)] \times 100\%$$

I = Intact Plasma Membrane

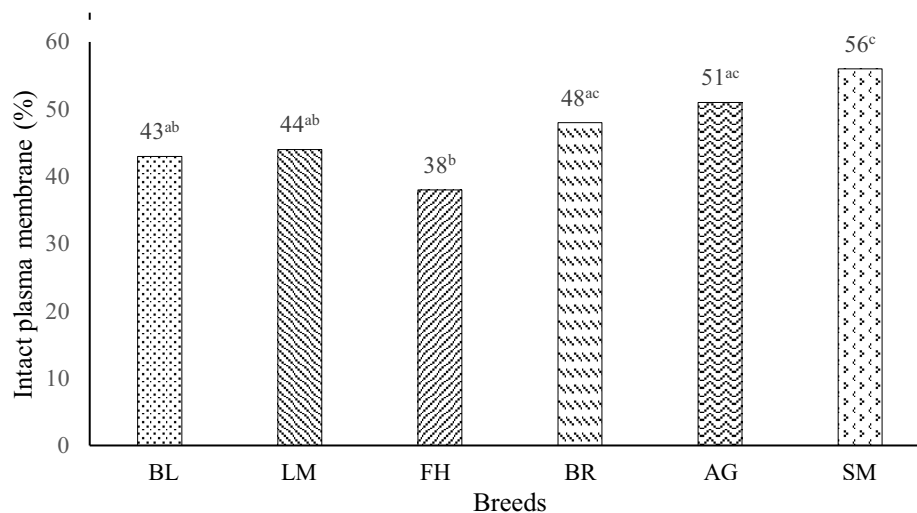
C = Total Circular Tail Sperm

S = Total Straight Tail Sperm

3. Results and discussion

3.1. Intact plasma membrane of different breeds

The Intact plasma membrane of different breeds presented in figure 1.



Note: Different superscript that follow the numbers in the diagram show significantly different ($P < 0.05$).

Figure 1. Intact plasma membrane of different breeds of cattle.

Figure 1 showed the quality of Intact Plasma Membrane. Spermatozoa with intact plasma membranes were characterized by a circular tail and damaged sperm were characterized by a straight tail. Cattle with the highest intact plasma membrane values were Simental cows and the lowest was FH cattle. The results of statistical analysis on the percentage of intact plasma membrane showed a significantly difference ($P < 0.05$) between cattle breeds. The percentage of intact plasma membrane in Simental cattle was significantly different ($P < 0.05$) compared to Bali, Limousine and FH. However, it is not significantly different from the Brahman and Angus cows. This proves that the different breeds of cattle can affect the intact plasma membrane level which will be related to the fertility rate.

Measurement of intact plasma membrane using HOST was used for functional evaluation of the integrity of the sperm membrane. Membrane integrity is a condition that shows the physiological function of the membrane which is maintained as a control for ion transport, so that fluids outside the cell cannot enter the cell. If the intact plasma membrane was damaged, the metabolic will be disrupted, ATP synthesis doesn't work normally and has fatal consequences for sperm, namely decreased motility and sperm survival. The varying percentage of intact plasma membrane occurs because of the biophysical and biochemical characteristics of the sperm membrane [9]. Spermatozoa must own the intact plasma membrane cause that is an absolute thing, the plasma membrane have an important role in organize all processes that happen in the cell [10]. The spermatozoa plasma membrane functions to protect spermatozoa organelles and transport electrolytes for spermatozoa metabolism [11]. Damaged to intact plasma membrane can affect the metabolism and physiological function of spermatozoa, causing spermatozoa to die [12]. The intact plasma membrane is a matter that greatly affects the function of the spermatozoa, the freezing and thawing processes can ruin the structure of the intact plasma membrane [13]. If the spermatozoa plasma membrane has been ruin, the spermatozoa metabolism will be disrupted and begin to lose its motility, the most fatal spermatozoa die [2]. Dead spermatozoa reduce the concentration of fertile sperm, and are also toxic to other living sperm [14]. Amin (1998) states that a complete (good) plasma membrane is absolutely necessary for spermatozoa in order for their survival to occur. Intact plasma membrane was damaged will cause the ability of spermatozoa to fertilize [15].

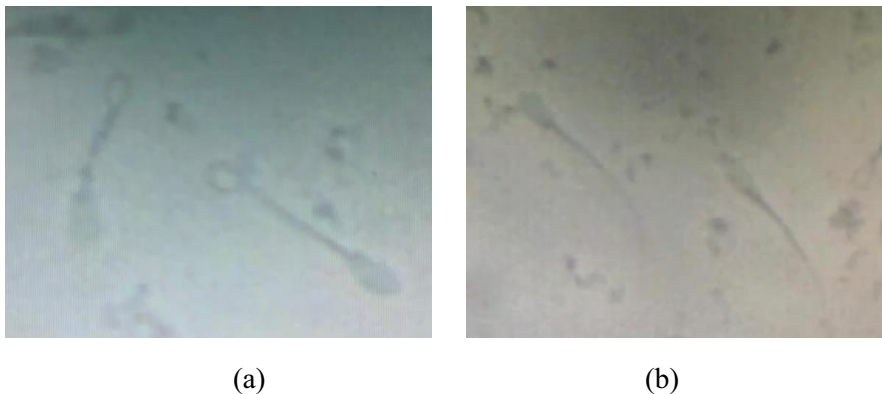


Figure 2. Intact Plasma Membrane; (a) Spermatozoa with circular tails
(b) Spermatozoa with straight tails.

Spermatozoa that have intact plasma membrane, after being exposed to a hypoosmotic solution using the hypoosmotic swelling test (HOS-Test) method, are characterized by a circular tail. Fafo et al (2016) stated that spermatozoa with a circular tail can occur because the medium that enters the cell is maintained by the intact plasma membrane [16]. Conversely, if the intact plasma membrane was ruin, it will be indicated by the spermatozoa tail remaining straight when exposed to a hypoosmotic solution because the plasma membrane that is no longer intact is unable to maintain the medium that has entered the cell (figure 2). There is damage to the plasma membrane due to the flow of water into the spermatozoa so that the plasma membrane can no longer maintain the osmotic balance [17].

Spermatozoa that have intact plasma membranes after being given the HOS solution, their tails look circular. This situation is caused because in cells that have intact plasma membranes, the solution that enters the cell can no longer get out so that the pressure inside the cell increases and the spermatozoa appears to have a circular tail. Conversely, in cells that have a damaged plasma membrane, the solution that enters the cell will come out again so that the spermatozoa tail looks straight. The plasma membrane contains high amounts of unsaturated fatty acids which are easily oxidized. This situation causes the spermatozoa membrane to be easily damaged by peroxidation [18].

4. Conclusion

It is concluded that differences in cattle breeds can affect the level of the intact plasma membrane. The intact plasma membrane level in Simental cattle has the highest value than other breeds and the lowest FH cattle.

References

- [1] Toelihere R M 1993 *Inseminasi Buatan pada Ternak* (Bandung: Penerbit Angkasa)
- [2] Yulnawati and Setiadi M A 2005 Motilitas dan keutuhan membran plasma spermatozoa epididimis kucing selama penyimpanan pada suhu 4°C *Media Kedokteran Hewan* **21**
- [3] Rodriguez-Martinez H 2003 Laboratory semen assessment and prediction of fertility: still utopia? *Reprod. Domest. Anim.* **38** 312–18
- [4] Ruiz-Sanchez A L, O'Donoghue R, Novak S, Dyck M K, Cosgrove J R, Dixon W T and Foxcroft G R 2006 The predictive value of routine semen evaluation and IVF technology for determining relative boar fertility *Theriogenology* **66** 736–48
- [5] Gadea J 2005 Sperm factors related to in vitro and in vivo porcine fertility *Theriogenology* **63** 431–44
- [6] Correa J R and Zavos P M 1994 The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane *Theriogenology* **42** 351–60
- [7] Neild D, Chaves G, Flores M, Mora N, Beconi M and Agüero A 1999 Hypoosmotic test in equine spermatozoa *Theriogenology* **51** 721–27
- [8] Zou C X and Yang Z M 2000 Evaluation on sperm quality of freshly ejaculated boar semen during in

- vitro storage under different temperatures *Theriogenology* **53** 1477–88
- [9] Sukmawati E, Arifiantini R I and Purwantara B 2014 Freezing capacity of sperm on various type of superior bulls. *JITV* **19** 168–75
- [10] Rizal M, Toelihere M R, Yusuf T L, Purwantara B and Situmorang B 2003 Kualitas semen beku domba garut dalam berbagai konsentrasi gliserol *Jurnal Ilmu Ternak Veteriner* **7** 194–99
- [11] Salmah N 2014 *Motilitas, Presentase Hidup dan Abnormalitas Spermatozoa Semen Beku Sapi Bali pada Pengenceran Andromed dan Tris Kuning Telur* Skripsi (Makassar: Fakultas Peternakan Universitas Hasanuddin)
- [12] Butarbutar E 2009 *Efektifitas Frekuensi Exercise terhadap Peningkatan Kualitas Semen Sapi Simmental* Skripsi (Medan: Fakultas Pertanian, Universitas Sumatra Utara)
- [13] Gordon I 2005 *Reproductive Technologies in Farm Animals* (Wallingford: CABI Publishing)
- [14] Arifiantini I, Yusuf T L and Yanti 2005 Kaji Bidang Semen Beku Sapi Frisien Holstein Menggunakan Bahan Pengencer dari Berbagai Balai Inseminasi Buatan di Indonesia *Animal production* **7** 168–76
- [15] Amin M R 1998 *Efektifitas Plasma Semen Sapi dan Kerbau dari Berbagai Pengencer dalam Meningkatkan Kualitas Semen Beku Kerbau Lumpur* Graduate Thesis (Bogor: Program Pasca Sarjana Institut Pertanian Bogor)
- [16] Fafo M, Hine T M and Nalley W M 2016 Pengujian efektivitas ekstrak daun kelor dalam pengencer sitrat- kuning telur terhadap kualitas semen cair Babi Landrace. *Jurnal Nukleus Peternakan* **3** 184–95
- [17] Jeyendran R S, Van der Ven H H, Perez-Pelaez M, Crabo BG, Zaneveld L J D 1984 Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics *J. Reprod. Fertil.* **70** 219–28
- [18] Herdis 2017 Fresh semen characteristic of garut rams fighting type at three semen collection time *Zoo Indonesia* 2017 **26** 8–19